Controlling Lethal Browning of *Hemarthria compressa* Tissue Cultures

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Introduction

Hemarthria compressa is an important warm-season forage grass for use in Southwest China. However, due to poor seed set, it is propagated by vegetative cuttings of stolons, rhizomes, and nodal sections. The *in vitro* propagation of *H. compressa* is still faced with difficulties including blackening or browning of tissues prior to culturing due to the oxidation of phenolic compounds by polyphenolic oxidase enzyme present in excised tissue (Yang *et al.* 2008). The objectives of the study were to investigate possible means of successful initiation of cultures through elimination of phenolic browning.

Materials and methods

Shoot tips from clonal *H. compressa* cv. Ya'an were obtained from fields of established plantings at Sichuan Agricultural University, Sichuan Province, China. Explants were stored in antioxidant solution of 150 mg/L citric acid and 100 mg/L ascorbic acid prior to surface sterilization. Surface sterilization was achieved by soaking explants in 10% (w/v) sodium hypochlorite solution containing 0.01% Tween 20 wetting agent for 20 minutes and rinsing three times with sterile distilled water. All explants were initially cultured on MS (Murashige and Skong 1962) medium with 2, 4-D (1.0 mg/L) and 3 %(w/v) sucrose and solidified with 0.58 (w/v) agars for callus subculture. The media *p*H was adjusted to 5.8 before autoclaving. Cultures were inoculated in the dark under controlled culture room conditions of 28 ^{o}C using air-conditioners (Gree kFR-50LW). Relative humidity was maintained at (50-60%) using a humidifier (ZR10, China).

The following treatments were evaluated as means for browning control: (1) explant orientation: horizontal or vertical on the medium surface; (2) illumination: shoot tips and young leaves were either incubated under 14 hours day length or complete darkness and; (3) preincubation soaking in activated charcoal (AC) where shoot tip explants were soaked in solutions of different concentrations of AC (0, 1, 2, 3 g/L) for 2, 5, 15, or 25 hours. Data were recorded after four weeks.

A completely randomized design was used for all experiments, where 10 to 15 replications were employed per treatment. Visual recordings were set for media browning and explant vigor. The browning scale was 1 to 10, where 1 represented the least and 10 the maximum browning intensity. Vigor scale was 1 to 5, where 1 = poor, 2 = fair, 3 = good, 4 = very good, and 5 = excellent. Standard errors of means were analyzed using SAS 9.0 software.

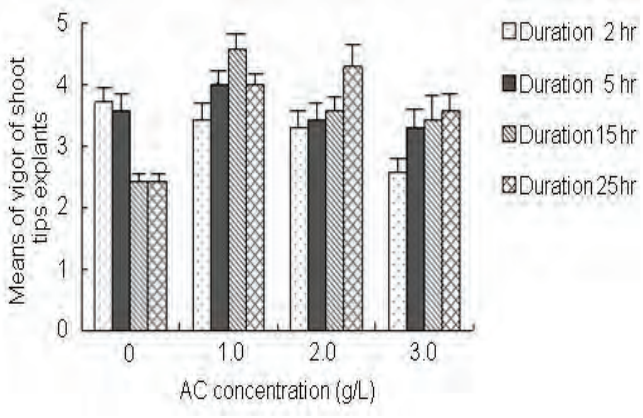

Figure 1. Means of vigor of shoot tip explants as affected by pretreatment with 0, 1, 2, and 3 g/L of activated charcoal for 2, 5, 15, or 25 hours.

Results and discussion

Vertical placement of explants resulted in enhanced culture vigor and the least browning (Table 1). Horizontal orientation resulted in two contact sites with the media surface in which there is a higher chance for phenolics to leak from the two cut surfaces of the explants to the medium. Dark incubation of shoot tips reduced media browning but failed to support higher survival or vigor compared to illumination. This may be due to the lengthy (four-week) dark period.

In the subsequent test for activated charcoal (AC) optimization, presoaking in a solution of 1.0 g/L AC for 15 hours was found to be the best (Fig. 1). However, in detergent-free presoak solution, the chance of explant contamination increased with time, and because time adds to labor costs, thefive-hour period was chosen based on the observed high vigor level.

Conclusion

Vertical positioning of shoot explants and dark incubation combined with presoaking explants in a solution of

Table 1. Performance of *H. compressa* tissue cultures in response to explant orientation and illumination treatments.

Orientation	Survival (%)	Vigor	Browning intensity
Horizontal	42.6±0.58b	1.84±0.21b	6.54±0.42a
Vertical	75.4±1.12a	3.37±0.28a	3.52±0.51b
Illumination			
Light	82.7±0.31a	2.94±0.28a	3.01±0.23a
Dark	50.2±0.84b	1.45±0.41b	2.24±0.22b

*Values within a column followed by a different letter are significantly different at $P<0.05$.

1.0 g/L activated charcoal can be used to counteract the adverse effects of phenolic browning of *H. compressa* cultures.

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